

Research Article



Evaluation of Physiochemical Properties and Acute Toxicity Studies of *Miruthar Singi Parpam*

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ABSTRACT

Siddha medicines which are popular in southern parts of India have certain quantity of heavy metals, but the heavy metals used in these medicines are completely transformed into inert compounds by proper purification and preparation methods before being prescribed as medicine. However, one of the problems in the acceptance of the siddha medicines is the lack of standard quality control profiles. The present study was designed to investigate one such siddha formulation *Miruthar singi parpam* (MSP). It is a herbo-metallic formulation of Siddha medicine and has been used for various ailments. It consist Sulphide of Lead, Galena, processed with fresh leaf juice of *Datura metal Linn*. The chemical finger print was engaged by using modern technique- Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The particle size was assessed by Scanning Electron Microscope (SEM). To evaluate its safety, acute oral toxicity studies were performed following organization for economic co-operation and development (OECD) test guidelines 423. Acute oral toxicity study was carried out in male and female Swiss albino mice and the animals were treated more than ten times with the therapeutic dose of *Miruthar singi parpam* and they were observed for toxic signs for 14 days. The SEM image of MSP showed numerous particles that ranged between 2-5 μm denoting a better bio-availability. The ICPOES analysis revealed that the heavy metals such as Arsenic, Mercury and Cadmium were below detectable limits thereby proving MSP to be safe. Acute oral toxicity study showed no lethality in experimental animal with ten times of therapeutic dose. Thus, *Miruthar singi parpam* (MSP) proves to be a safe medicine.

Keywords: *Miruthar singi parpam*, siddha formulation, SEM, acute oral toxicity.

INTRODUCTION

Siddha system of medicine is a great heritage of India. Siddha is the holistic system of codified life style health care perfected thousands of years ago in Tamil speaking peninsular India. It is developed by the siddhars, the ancient supernatural spiritual saints of India. The Siddha system of medicine uses a fascinating combination of herbs, minerals, metals to promote good health and longevity. Siddha system is an earliest medical science that stress on positive health, a harmonious blending of physical, mental, social, moral and spiritual welfare of individuals.¹

This system works on the basic concept that a healthy soul can only be developed through a healthy body. In today's world human beings are bordered by loads of diseases and one such condition is hemorrhoids. It is excruciating the human beings by its symptoms. Piles are the varicosities of the veins of the hemorrhoidal plexus.² In India the prevalence of hemorrhoids are very high. Country dig up second place in prevalence rate. Most recent statistical analysis report explains 40,723,288 populations are affected by piles out of 1,065,070,607.³ According to WHO, 40% of the people in the world suffer from hemorrhoids. In India approximately 80% of the sufferers are in the age group of 21-50 years. Hemorrhoids are common in both men and women.⁴

Major causes for the disease are Portal hypertension and chronic constipation. Due to the ecological changes and food habits, most of the populations today are affected by hypertension and constipation.⁵

In siddha system of medicine *siddhar's* who are spiritual scientists have explained briefly about the Hemorrhoids and have also provided wonderful formulations for the same. '*MIRUTHAR SINGI PARPAM*' (MSP) is one of the important formulation indicated specially for Hemorrhoids and Leucorrhoea.

The quality of traditional medicine is the sum of all factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product.

Standardization and safety of drug is the Major concern of treatment which demands evaluation whereas efficacy needs just a validation.⁶ This study will provide scientific validation of safety and efficacy of '*MIRUTHAR SINGI PARPAM*'.

MATERIALS AND METHODS

Procurement and authentication of raw drugs^{7,8}

The drugs were appropriately collected from the market in Chennai and were authenticated by, SCRI (Siddha Central Research Institute), Arumbakkam, Chennai. *Miruthar Singi* was powdered well and ground with fresh



leaf juice of *Datura metel* Linn. gradually for 12 hrs. It was made into small cakes of uniform size and dried under sunlight.

Preparation Process⁹

Step-1

Allium sativum tuber was ground well into a paste form with the latex of *calotropis*.

Step-2

The grinded paste was applied over the purified *Miruthar singi*. And then it was placed in a mud pot and closed with

a suitable mud lid. The closed edges were covered with 2 folded clay cloths and dried.

Step-3

It was then subjected to Pudam process using 25 palam (875 gm) of Varati (cow dung cake).

The product was again subjected to step 1 and step 2 and then again step 3, the pudam process, but it was heated with 22 palam (770 gm) of Varati (cow dung cake). This process was repeated for 5 times [In every pudam process the amount of cow dung cake must be reduced up to 3 palam (105 gm)].



Figure 1: Photos of of raw *Miruthar singi*, purified *Miruthar singi* and *Miruthar singi parpam*

Physicochemical Analysis

Color

About 50g of *Miruthar singi parpam* was taken in a clean glass beaker and tested for its color by viewing again a white opaque background under direct sunlight.

pH

The pH of *Miruthar singi parpam* was estimated as per the method prescribed in Indian Standard (IS) – 6940 (1982). One gram of the *Miruthar singi parpam* was taken in a 100ml graduated cylinder containing about 50ml of water and filled up to the mark with water.

The cylinder was stopped and shaken vigorously for two minutes and the suspension was allowed to settle for an hour at 25 to 27°C.

About 25ml of the clear aqueous solution was transferred into a 50ml beaker and tested for pH using DIGISUN digital pH meter (DIGISUN Electronics, Hyderabad, India.)

Determination of Ash Value

2 grams of *Miruthar singi parpam* is weighed accurately, added in tarred platinum or silica dish and incinerated at a temperature not exceeding 450°C until free from carbon, then cooled and weighed. The percentage of ash is calculated with reference to the air dried drug.

Water Soluble Ash

To the gooch crucible containing to the total ash, added 25 ml of water and boiled for 5 minutes after that collected the insoluble matter in a sintered glass crucible or on ash less filter paper. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450° C.

The weight of the insoluble matter is subtracted from the weight of the ash and the difference of the weight represents the water soluble ash.

The percentage of water soluble ash is calculated with reference to the air dried drug.

Acid Insoluble Ash

The ash is boiled 5 minutes with 25 ml of 1:1 dil HCL. The insoluble matter is collected in gooch crucible on an ash less filter paper and washed with hot water and then ignited.

Cooled in desiccators and weighed. The percentage of acid insoluble ash is calculated with reference to the air dried drug.

Loss on Drying

Five grams of *Miruthar singi parpam* is heated in a hot oven at 105°C to constant weight and the percentage of loss of weight was then calculated from it.

Qualitative Analysis

Preparation of Extract

5 gm of *Miruthar singi parpam* is weighed accurately and placed in a 250ml clean beaker and 50 ml of distilled water is added to it. Then it is boiled well for about 10 minutes.

Then it is cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water.

Qualitative and quantitative analysis was done following the procedure detailed in pharmacopoeia standards for Ayurvedic formulation.¹⁰⁻¹²

Quantitative Analysis^{13,14}

Particle size determination by Scanning Electron Microscopy

Sample Preparation

Sample preparation can be minimal or elaborate for SEM analysis, depending on the nature of the samples and the data required. Minimal preparation includes acquisition

of a *Miruthar Singi Parpam* that will fit into the SEM chamber. And then it should be analyzed.

ICP OES Methodology^{15,16}

Solids cannot be analyzed directly. 200mg of substance was taken and dissolved in 5mL of 5% of water or aqua regia or whatever acid to make 100mL of final solution. Ideal concentration is around 100 ppm of the element of interest. A minimum solution volume of 25 ml is necessary for analysis. In ICP intensity of light emitted when the sample is sprayed or aspirated into argon plasma was measured at different wavelengths. The intensity of light at a given wavelength will be proportional to a particular elemental ion concentration. The intensity was calibrated with known standard concentration. For accurate quantitative result, it is necessary to simulate the sample matrix condition with that of the standard. Each element generally will have many emission lines and the sensitivity is different for each of this wave length. When more than one element is present it is quite common that some emission lines interfere due to overlapping.

Toxicity studies

Animals

The study protocol involving animals were approved by Institutional Animal Ethical Committee (IAEC), National Institute of Siddha with the experimental protocol number **1243/ac/09/04-2010** prior to the initiation of the study. Test animals were obtained from the animal House of the King Institute, Chennai. Swiss albino mice of either sex weighing about 20-25 gms were selected and kept under standard laboratory conditions.

The temperature in the experimental animal room was maintained at 22 °C (\pm 3°C). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%.

Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. The animals were acclimatized 7 days prior to study. The animals had free access to water and standard pellet diet (Sai Meera Foods Pvt. Ltd, Bangalore).

Acute oral toxicity study for 14 days

The Acute Oral Toxicity study was performed in accordance with OECD test guideline-423.¹⁷ Young, healthy Swiss albino mice of either sex have been randomly selected for studies.

The animals were treated with the dose of ten times therapeutic dose level. The animals were weighed before giving the test drug. Animal dose was calculated by the following formulation [(Therapeutic dose x (0.0018) x 10], since the therapeutic dose is 65mg/kg, so the 10 times dose was calculated as 1.17 mg/kg. and hence the test group animal was treated with 1.17 mg/kg of the test drug.

Table 1: Animal grouping for acute oral toxicity studies

S. No	Group	No. Of Mice
1.	Control group (Vehicle)	6 (3 males, 3 females)
2.	Test group (Therapeutic dose x 0.0018 x 10)	10 (5 males, 5 females)

The test group was treated with a single oral dose of 1.17 mg/kg *Miruthar singi parpam* suspended in Tween 80 solution (10%), with uniform mixing and was administered to the mice in a single oral dose by using oral gavage. The control group received an equal volume of the vehicle. After the test drug has been administered, food was withheld for further 3 hours in rats. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days, except when they needed to be removed from the study and humanely killed for animal welfare reasons.

All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

RESULTS AND DISCUSSION

Physicochemical Properties of *Miruthar Singi Parpam*

The product was found to be a light brown solid, with the pH range of 7.0-7.3. The water extract gave a maximum yield of 49%w/v. Physicochemical parameters of the MSP are shown in Table 2. The loss on drying value obtained is an indicative of amount of moisture content present in the drug. The loss on drying at 105°C in MSP was found to be 6.2%. The total ash value was 9.52% and its an indicative of total amount of inorganic material after complete incineration. The acid insoluble ash value of MSP was 0.95% and its an indicative of silicate impurities, which might have arisen due to improper washing of drug.¹⁸ Analytical result showed that water soluble ash, moisture content and foreign organic matter were found to be 5.5%, 6.2% and 4.2% respectively. The percentage yield of alcohol and water extractive value (5 and 9.45) helped us in determining the amount of active Constituents found in the preparation.

The qualitative inorganic analysis showed the presence of sulphate, chloride, phosphate, carbonate, sulphide, nitrate, nitrite, fluoride, nitrate, oxalate, borate, lead, copper, aluminium, zinc, iron, calcium, magnesium, ammonium, potassium, sodium and mercury. The organic components analyzed were, starch, alkaloids, reducing sugar, oxyquinole epinephrine, pyrocatechol, aliphatic amino acids and meconic acid, apomorphine, salicylate, resorcinol, morphine, phenol, cresol and hydro quinone.



Table 2: Physicochemical analysis of *Miruthar Singi Parpam*

S No.	Physicochemical character	Values obtained (% w/w)
1	Total ash value	9.52
2	Acid insoluble ash	0.95
3	Water soluble ash	5.5
4	Moisture content	6.2
5	Foreign organic matter	4.2
6	Alcohol soluble extractive	5
7	Water soluble extractive	9.45
8	Loss of weight at 105°C	6.2

Table 3: The constituent of raw *Miruthar singi*, purified *Miruthar singi* and *Miruthar singi parpam*

Compound	Raw <i>Miruthar singi</i>	Purified <i>Miruthar singi</i>	<i>Miruthar singi parpam</i>
Lead	✓	✗	✗
Calcium	✓	✓	✓
Sulphide	✓	✓	✓
Iron	✓	✓	✓
Reducing sugar	✓	✓	✓
Starch	✓	✓	✓

Where ✓ - Presence ✗ - Absence

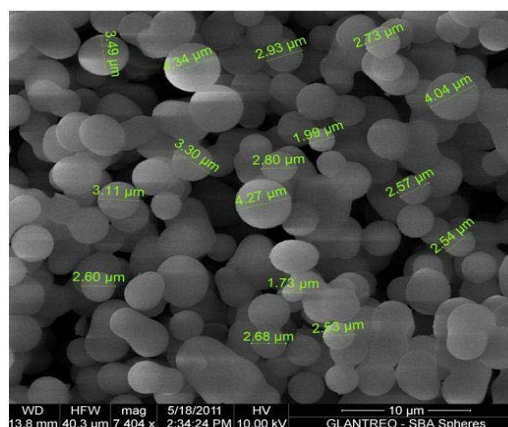
Result of High Resolution Scanning Electron Microscope (HR-SEM)

The SEM analysis of *Miruthar singi parpam* showed the even distribution of spherical shaped particles in a size range of 2 to 5 µm and the surface was found to be smooth. SEM picture of MSP is shown in Figure 2. Hence the MSP, which is prepared biologically, contains micro

Table 4: Inductively Coupled Plasma-Optical Emission Spectroscopy for the of raw *Miruthar singi*, purified *Miruthar singi* and *Miruthar singi parpam*

S. No.	Elements	Wavelength(nm)	Range of Observation		
			Raw <i>Miruthar singi</i> (ppm)	Purified <i>Miruthar singi</i> (ppm)	<i>Miruthar Singi parpam</i> (ppm)
1	Arsenic (As)	193.696	BDL	BDL	BDL
2	Calcium (Ca)	317.933	15.86	14.44	10.33
3	Cadmium (Cd)	226.502	BDL	BDL	BDL
4	Mercury (Hg)	253.652	BDL	BDL	BDL
5	Potassium (K)	766.49	47	17.13	9.46
6	Sodium (Na)	589.592	1.339	1.22	2.13
7	Phosphorus(P)	213.617	14.237	1.101	1.742
8	Lead (Pb)	230.204	39.99	19.24	7.35
9	Sulphur (S)	181.975	114.231	217.079	2.134
10	Iron (Fe)	238.204	1.653	1.24	1.04

particles that easily penetrate the cell and interact with cellular molecules and enhancing fast pharmacological action at target sites.¹⁹ As the particle is in micro size, a low dose of the drug is more than enough to treat diseases.

**Figure 2:** SEM analysis of *Miruthar singi parpam*

Inductively Coupled Plasma-Optical Emission Spectroscopy (ICPOES)

The ICPOES analysis of MSP in Table 4 reveal that the heavy metals such as Arsenic, Mercury and Cadmium are below detectable limits (BDL) and Heavy metal lead found to be 7.35 ppm. According to WHO & FDA reports the permissible limit of heavy metals for safe clinical practice is arsenic (10 ppm), lead (10 ppm), cadmium (0.3 ppm) and mercury (1 ppm). It also have physiologically important minerals such as Na, K, Ca, P and Fe.

Result of Toxicity Studies

Acute oral toxicity studies for 14 days with the 10 times the dose of therapeutic dose showed no mortality in mice. There was no significant difference in the body weight and the behavioral changes that are tabulated in Table 5.

Table 5: Acute toxicity study (Behavioral changes & Mortality)

S. No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	Control	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	n	N	↑
2	Test	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	n	N	↑

1. Alertness 2. Aggressiveness 3. Passivity 4. Grooming 5. Gripping strength 6. Touch responses 7. Restlessness 8. Tremors 9. Convulsion 10. Pain response 11. Righting reflex 12. Pinna reflex 13. Corneal reflex 14. Pupillary size 15. Lacrimation 16. Salivation 17. Urination 18. Skin color 19. Mortality 20. Body weight. (+) =Positive response. (-) = Negative response, (↑) = Increased, N = Nil, n-normal

CONCLUSION

Based on the above study, the Siddha herbo-mineral preparation of MSP is non toxic in nature shown by acute toxic study even when it is consumed more than ten times of its therapeutic dose. The standardization of the drug was evaluated by chemical characterization with heavy metal analysis, quantitative and qualitative analysis of elements and determination of particle size by ICP-OES and SEM respectively. The SEM picture had shown the presence of micro particle of size 2-5µm in the drug *Miruthar singi parpam*.

ICP-OES reveals that MSP has heavy metals like, As, Cd, Pb and Hg and was below detectable level. It also has physiologically important minerals like Na, K, P, Fe and Ca. This reveals the safety of the drug.

Hence, it is concluded that MSP is a kind of micro medicine which favors the advantages like, increased bio availability, better absorption and non toxic in nature. Even at minimum dose, MSP can produce maximum therapeutic effect. In this study, we evaluated the physicochemical characteristics and acute toxicity effects of MSP. In future, this work can be extended further for the toxicological studies at sub acute and chronic levels.

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